A SURVEILLANCE AND CONTROL PROGRAM FOR BLUE TONGUE

by

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ABSTRACT

The viral disease blue tongue has a relatively low prevalence in New York. It is thought that New York born-and-reared cows do not contract the disease. Since the identification of a blue tongue diseased cow in a herd results in a quarantine of the herd, and perhaps death of the cow, rather serious economic losses can be incurred. Therefore, there is pressure to eradicate the disease. Sampling procedures are presented for testing the hypothesis of zero incidence of blue tongue in New York born-and-reared cattle and for estimating an upper bound on prevalence of the disease. A control and surveillance program is described.

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1. INTRODUCTION

Blue tongue (BT) is a viral, insect-borne infectious disease of domestic and wild ruminants. Transmission of BT virus requires a biological or mechanical vector. In cattle, the virus may also be transmitted through semen from infected bulls. Outbreaks of BT generally occur in the late summer and early fall, and topographically are associated with rivers, creeks or marshlands. The time and location of the outbreaks coincide with the prevalence of the gnat insect vector, *Culicoides variipennis.* At present, there are five known serotypes of the virus in the United States.

BT is primarily a disease of sheep and all breeds are susceptible although to variable degrees. Following the detection of the disease in sheep, BT infections have been detected in cattle, goats and various wild ruminants, such as white-tailed deer, elk and bighorn sheep. The disease in cattle is generally subacute with few overt clinical signs. Illness in acutely infected cattle herds varies greatly but in the typical outbreak less than 10% of the cattle are visibly sick. The clinical signs include one or more of the following: fever, excessive salivation, edema of the lips, nasal and/or ocular discharge, ulcers on the tongue, dental pad and muzzle, and stiffness and laminitis in all four limbs (sometimes severe inflammation of the coronary band and sloughing of the hoof). If infection occurs early in pregnancy this disease may cause abortion or congenital deformities. Experimentally infected cattle rarely show any clinical signs even though the virus persists for 20 to, in a few instances, more than 100 days. Infected cattle develop serum antibodies within two or three weeks following infection, but virus may be isolated from animals which have

serum antibodies. In the United States, it is believed that cattle may be the major reservoir of BT virus because of the occurrence of chronically infected animals. The role of wild ruminants in maintenance of BT virus is largely unknown. The major direct economic impact of the disease in the cattle industry may be the production of abortions and congenital effects.

The diagnosis of BT disease can be made by the isolation of the virus or by the demonstration of antibodies in the blood. Although virus isolation is the most definitive diagnostic test, the procedure is time consuming and expensive. Standard tissue culture techniques are inadequate for the consistent isolation of BT virus. Alternative procedures such as sheep inoculation and/or the intravenous inoculation of 10-day-old embryonated eggs are considered the most sensitive test for virus isolation. However, few diagnostic laboratories are equipped to do routine BT isolations and most clients cannot afford the high fee for the tests. To test for antibodies in the blood, two tests, the agar gel immunodiffusion (AGID) and the serum neutralization (SN) tests, are currently employed. The AGID test is simple and inexpensive, but less sensitive. The SN test is sensitive but expensive because of the need to run multiple tests for the different BT virus serotypes.

BT disease has never been reported in New York State. Additionally, serological data suggests that the infection of New York cattle is an extremely rare event, if it occurs at all. This is true in most northeastern states, in contrast to the high frequency of BT disease in the rest of the nation. A recent 1983 USDA survey of prevalence revealed only 0.8% positive reactors (four animals out of 480 sampled at slaughter) in New York State. Unfortunately, because of the sampling type for this survey, there is no available information indicating whether these positive

reactors were native to New York. Based on this information, the direct economic loss due to BT disease in New York State is virtually nil. However, the indirect loss due to continuous denial of export markets for cattle, cattle semen and embryos, could be substantial if the BT-free status of New York State is not adequately documented with reliable scientific data.

A number of sample survey designs are presented. The goals attainable with each are discussed. A surveillance sampling design is described. Procedures for maintaining a BT-disease-free herd are given. The ideas of group testing and double sampling are presented in light of the conditions of the surveillance program.

2. SAMPLE DESIGNS

Many possible sample designs could be constructed to study various aspects of the disease blue tongue. Various goals could require different designs. Some possible designs and related goals are discussed below.

Slaughterhouse – A stratified random sample of animals slaughtered would produce a prevalence estimate of animals slaughtered in New York. A list of slaughterhouses would provide a sampling frame. The probability of selecting a slaughterhouse would be proportional to the number of animals slaughtered per year by a slaughterhouse and a specified proportion of animals would be sampled. A proportional probability sample would result.

In order to compare prevalence rates of blue tongue virus (BTV) in New York born-and-reared cows with cows not born and reared in New York, it would be necessary to trace the origin of a cow. This would appear to be a difficult if not impossible task.

Point of sale – Regional sales places exist throughout New York State. The majority of cows from dairy herds are sold through these sales places. A list of these sales places would form a sampling frame. Again using a proportional probability sample, a random sample of sales places with selection probability proportional to number of animals sold per year, would be obtained. A specified proportion of animals sold would be sampled and tested for BTV. If the results were made available prior to sale of an animal, this could result in a lower sale price. Owners then would not allow blood to be taken from their animals. In order to compare prevalence for New York born-and-reared cows and for others, it would be necessary to trace the origin and history of a cow. For cows that have appeared in several herds and/or in several states, this could be a difficult task.

There is some hope that the origin and herd history of a cow can be obtained.

Sampling herds – A list of dairy herds (e.g., from DHIA records) would provide a sampling frame for obtaining prevalence estimates. A "closed herd" is defined to be a herd into which no animals are allowed to come in from outside the herd. An "open herd" is one in which other animals are brought into the herd. The incoming animals may be from out of state or from other New York dairy herds.

To test the hypothesis that BTV is not found in New York born-and-reared cows, the following sample design is suggested. First prepare a sampling frame of open and closed herds using a telephone survey, mailed questionnaire, or other relatively cheap method of surveying all New York State dairy herds. The questions asked would be of the form:

i) How many cows in the herd?

ii) How many cows are four years of age or older?

iii) How many cows were reared in this herd?

iv) How many cows are from other herds in New York?

v) How many cows are from out of state?

vi) How many cows are of unknown origin?

From this survey, either a simple random sample or a proportional probability sample of 500 closed and 500 open herds would be selected. Four cows four years or older would be randomly selected from each of the 500 closed herds, resulting in a sample of 2000 cows. From the open herds, two New York born-and-reared cows and two out-of-state cows would be randomly selected from each of the 500 open herds. This again would result in a sample of 2000 cows. The 500 closed herd survey would be conducted first.

The rationale for the above sample survey design is as follows. As stated in the introduction, blue tongue disease has never been reported for New York born-and-reared cows. To test the hypothesis that no New York born-and-reared cows have blue tongue, the sample survey design on closed herds would be conducted first. If any cows are found to have blue tongue disease, the hypothesis is disproved. The reason for selecting cows four years of age or older is that this maximizes the probability of finding a diseased animal. The probability of a cow getting blue tongue disease is proportional to the number of years of exposure. Four years of age or older was selected because the average age of a herd is thought to be about four. Also, if cows four years or older do not have blue tongue, it is safe to say that younger animals in the herd do not have the disease. If cows in the closed herds are found which have blue tongue, then the prevalence estimate obtained would refer to the population of cows four years of age or older. It would be an upper bound on prevalence for cows in closed herds.

The sample survey design on open herds would allow an answer to the question "Are prevalence rates in New York born-and-reared and other cows different?" This again would refer to the population of cows four years of age or older in open herds and would be an upper bound on the difference in rates.

Sample size considerations and age of cow were selected so as to be able to detect low rates and to compare small differences in rates. The only estimate of prevalence, 0.8%, available is based on scant evidence, 4 diseased animals out of 480 examined. This no doubt relates to older animals as they are the ones most frequently found in slaughterhouses. If this were the rate, then in 2000 animals one would expect to find

.008(2000) = 16 animals with the disease. As a first approximation, it might be expected that the number of animals diseased follows a Poisson distribution. The mean and variance are equal in a Poisson. Using a normal approximation for a 95% confidence interval, $16 \pm 2\sqrt{16}$, the lower limit on diseased animals would be eight and the upper 24. A 99% confidence limit would give limits of $16 \pm 2.6\sqrt{16}$, or 5 to 27. Thus, if the prevalence was 0.8% we could be fairly certain to obtain at least five diseased cows in a sample of 2,000 cows. The actual distribution of numbers probably follows a negative binomial or some type of contagious distribution more closely than a Poisson, as the probability of finding two diseased cows in the same herd is higher than for two cows in different herds. That is, there is a "clumping" or "contagion" of diseased animals and diseased animals are not distributed with equal probability over all herds.

For the sample survey on open herds, it is suspected that the prevalence rate is much higher for non-born-and-reared New York State cows. This group also makes up a minority of all cows in New York. If this rate were 2.5%, say, then one would expect .025(1000) = 25 diseased cows in a sample of 1000 cows. If the proportion of diseased cows among New York born-and-reared cows were .2%, say, then the expected number of diseased cows would be 1000(.002) = 2. Using normal approximations, then $t = (25-2)/\sqrt{25+2} > 4$ which is a relatively large value for t. Hence, the sample size is more than sufficient to detect differences in rates of the above size.

In the open herds, any New York born-and-reared cows that are diseased could have been infected by the gnat insect vector or through use of blood-contaminated instruments. If the closed herds had zero prevalence but the open herds did not for native cows, then one could conclude that

the disease was transmitted through use of blood-contaminated instruments. The various methods of transmission of BTV requires further study.

A second stage of stratification may be desirable. The county or a region could be used as a first step in the stratification. The second level of stratification would be the herd as above. Proportional probability samples would be recommended in all cases for this study.

If any diseased animals are found in a herd, the herd is placed under quarantine under New York State regulations. It is recommended that for any herd in which diseased animals were found, that the entire herd be sampled immediately before reporting the diseased animals. The reason is to avoid the mistake of mistakenly placing a herd under quarantine, which would result in considerable economic loss to the farmer.

Surveillance sample design – In any ongoing disease detection program it is essential to resample at various intervals through time. A surveillance program is of this nature. Robson (1959) has devised such a plan (see Appendix). Another such plan has been devised by Federer *et al.* (1985) for *Bovine leucosis.* The group testing ideas discussed in the latter paper would have considerable relevance here, as the prevalence of blue tongue is quite low. To test a single pool from a herd, a test must be quite sensitive. One such test would be to inject the pooled sample into a sheep. Several injections may be necessary. The cost per herd to ascertain disease-free status would not be exorbitant, i.e, the cost of one sheep. However, it would be prohibitively costly if one sheep had to be used for each cow in the herd. For the individual samples or for pools of small numbers of animals, a less expensive test should be used. If sensitive and relatively inexpensive laboratory tests for BTV were avail-

able, they should be used in place of the above biological sheep assay. If the sheep with blue tongue could be marketed, the cost of a sheep assay would be minimal.

3. EPIDEMIOLOGICAL ASPECTS

In establishing a surveillance and control procedure for BT the following factors need to be considered:

(i) Disease transmission – It is not known if or how frequently BT can be transmitted from sheep or deer to dairy cattle. Also, it is not known how frequently, if at all, BT can be transmitted from cow to cow through blood-contaminated instruments such as needles, dehorning, castrating, or other instruments. Also, can BT be transmitted through animal contact? Or, does all transmission of BT occur through an insect vector?

(ii) Period when transmitted – It is thought that BT infection occurs irregularly throughout the year. Insect populations are larger and more active in spring, summer, and fall than in winter. It is expected that few insects would be found during the winter months except for perhaps a prolonged warm period. Population sizes of insects transmitting BT may vary from spring to summer to fall. The time when BT infections occur is important in setting up any surveillance and control program.

(iii) Incubation period – After an animal has been infected with BT virus, it takes a period of time for the disease to develop and manifest itself. If, for example, the disease was only transmitted in June, July, and August and the incubation period was six months, a surveillance every February or March would suffice. A once-a-year inspection is all that would be necessary.

As is evident from the above, information on the epidemiological aspects of BT is vital in determining when to reinspect a herd for BT. Incorrect timing would cause diseased animals to go undetected until the following inspection period. The diseased animals would be a source of infection for other animals in the herd. The period in which maximum titer occurs after infection should be used to determine the time of inspection. The higher the titer level the less likely it is to go undetected in laboratory analyses. This type of surveillance will allow detection of diseased animals in the shortest period of time. If it is known that the incubation period only lasts a certain number of months and that the animals can only become infected in summer, say, a quarantine could be lifted from a herd as soon as the diseased animals have been removed and no others allowed in. Knowledge about the above items is essential for determining the time and frequency of inspections and in establishing herd quarantine procedures. This must be accompanied by an active and effective control program of the insect transmitting the BT virus.

4. A SURVEILLANCE AND CONTROL PROGRAM FOR BLUE TONGUE

From what is now known (or suspected) about BT, it appears that reinspection of a herd on a one-time-a-year basis should be frequent enough in the early stages of the program. In areas that have been free of BT for two years, say, and with no immigration into the area, the inspection period may be lengthened to once every two years. If it turns out that no native New York cows become infected with BT, then the inspection period may be lengthened to once in four years, say, provided no non-native cows or cows from infected areas have entered the region in this period.

Blood samples from each and every cow in a herd will be taken and sent to the New York State Diagnostic Laboratory. An equal aliquot from each sample from a cow in a herd will be taken to form a single pool for the herd. A test will be conducted on this pooled sample to determine if any cows in the pool have BT. If the test is injection of blood into a sheep, it is recommended that several injections be made to assure that the sheep receives sufficient BT virus to become infected if any cow has BT. Alternatively, two sheep could be injected. If a precise ELISA test is available, perhaps one test on the pooled blood sample will suffice. These details need to be worked out.

If a test does not always detect the presence of one or more diseased cows in a herd of h animals, it is suggested that a second pool be made and tested. If p is the probability of not detecting the presence of one diseased animal in a pool of h samples, then the probability of failing to detect the presence of one diseased animal in two pools is p^2. For p small, p^2 is considerably smaller. If a greater probability of detection is desired, then use three independent pools and tests.

Once the pooled sample from a herd is positive, indicating the presence of one or more diseased cows, one of the group testing procedures described by Federer, Clark, and Dubovi (1985) should be used. The prevalence can be taken as 1/h to determine optimum group size for group testing procedure I. Also, either procedure II or III could be followed. These group testing procedures will keep the laboratory costs at a minimum.

The blood samples should be taken in the same manner as described by Federer, Clark, and Dubovi (1985). Field costs should be kept to a minimum as described by these authors.

5. MAINTAINING A HERD FREE OF BLUE TONGUE

Some recommendations for maintaining a herd free of BT follow:

i) All animals in the herd should be blood tested for the presence of Blue tongue virus (BTV) antibodies every 12 months, by a USDA-approved test. A positive animal identification (unique ear tag or other) is required in all animals, as well as information on age and dam identification.

ii) Blood samples for BTV testing should be collected from all animals between November 15 and April 1 of each year.

iii) All BTV positive animals should be immediately removed from the herd. Calves under 5 months of age that are positive for BTV should be retested at 8 months of age before deciding on their removal.

iv) Herd additions should be BTV negative animals, older than 8 months of age. Animals should be quarantined in separate facilities and entered into the herd after one negative blood test 30 to 45 days after arriving on the farm.

v) Sterile disposable needles should be used only on one animal, and then discarded immediately.

vi) A different disposable obstetric sleeve should be used in the palpation of each cow.

vii) Blood-contaminated instruments (dehorning, castrating, etc.) should be disinfected between animals.

viii) Routine methods for insect control should be employed.

ix) Artificial insemination should be used in the herd.

As more is learned about the nature of the disease blue tongue, it may be possible to adjust the suggested management procedures and time intervals between samplings in the surveillance program. The cost of maintaining a BTV-free herd could conceivably be decreased.